Anti-aquaporin-5 and anti-poly-U-binding-factor-60kDa protein antibodies in primary Sjögren's disease patients: preliminary data and correlation with disease activity and severity indices

S. Stano[1], F. Cacciapaglia[1], A. Rinaldi[1], M. Giannotta[1], E. Urgesi[2], D. Natuzzi[1], F. Iannone[1]

[1]Rheumatology Unit DiPReMeJ, University of Bari, Italy;
[2]Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK.

Abstract

Objective

In primary Sjögren's disease (pSjD), in addition to glandular inflammation and atrophy, functional secretion impairment may contribute to dryness. Altered protein distribution and antibodies against aquaporin-5 (anti-AQP5) and poly-U-binding factor 60kDa protein (anti-PUF60) have been reported in pSjD and may be specifically implicated in the glandular secretive processes. This study aimed to assess the occurrence of serum anti-AQP5 and anti-PUF60 antibodies and their correlations with clinical and laboratory features of pSjD.

Methods

Blood samples from pSjD patients and healthy donors (HD) were collected, and anti-AQP5 and anti-PUF60 antibodies were detected using an enzyme-linked immunosorbent assay. Differences between groups were evaluated using appropriate statistical tests, and odds ratios (OR) of high disease activity were assessed by multivariate stepwise backward multiple regression and adjusted for clinical covariates.

Results

Serum samples from 36 pSjD patients and 8 HD were analysed, and anti-AQP5 and anti-PUF60 antibody levels were not significantly different between groups. However, pSjD patients with high disease activity (n. 10) had significantly higher levels of anti-AQP5 antibodies compared to those with low-moderate disease activity ($p<0.001$). At logistic regression analysis, variables associated with high disease activity were anti-AQP5 (OR 128.9, 95% CI 2.7–615), C-reactive protein (OR 12.9, 95% CI 1.2–137.2), and C4 <10 mg/dl (OR 60, 95% CI 1.1–318.9).

Conclusion

Our pilot study confirms that anti-AQP5 antibodies may discriminate pSjD patients with high disease activity. These findings offer valuable clinical implications for managing pSjD patients, potentially identifying patients at high risk of glandular deterioration.

Key words

primary Sjögren's disease, anti-aquaporin-5, anti-poly-U-binding-factor-60, ESSDAI, ESSPRI

*Stefano Stano, MD**
*Fabio Cacciapaglia, MD, PhD**
Angela Rinaldi, MD
Maria Giannotta, MD
Eduardo Urgesi, MD
Dorotea Natuzzi, PhD
Florenzo Iannone, MD, PhD

**Contributed equally.*

Please address correspondence to:
Florenzo Iannone
U.O. di Reumatologia,
Dipartimento di Medicina di
Precisione e Rigenerativa (DiMePRe-J),
Università di Bari,
Piazza G. Cesare 11
70124 Bari, Italy.
E-mail: florenzo.iannone@uniba.it

Received on April 8, 2024; accepted in revised form on June 18, 2024.

ORCID iD
S. Stano: 0000-0001-5207-7913
F. Cacciapaglia: 0000-0001-7479-4462
E. Urgesi: 0000-0002-0458-1469
F. Iannone: 0000-0003-0474-5344

Competing interests: F. Iannone has received honoraria or consulting fees from Alfasigma, GSK, Novartis, Pfizer. The other authors have declared no competing interests.

Introduction

Primary Sjögren's disease (pSjD) is a systemic autoimmune disease characterised by hypofunction of the exocrine glands, including salivary and lachrymal glands. This causes dryness in different body districts, most commonly leading to xerostomia and xerophthalmia. The pathogenesis of this disease is multifactorial and remains poorly understood (1).

Phenotypic characterisation of different mononuclear cells infiltrating the affected tissues offers additional insights concerning cellular proliferation, survival, and migration mechanisms, antibody secretion and the potential of forming tertiary lymphoid structures, known as germinal centres (2). Specifically, chronic lymphocytic sialadenitis, predominantly consisting of $CD4^+$ T-lymphocytes and CD20+ B-lymphocytes infiltrating glandular tissue, promotes the maintenance of the inflammatory processes and the local expression of self-antigens. This activates B-cells and triggers the formation of specific autoantibodies, including SS-related antigen A (SSA), SS-related antigen B (SSB) but also rheumatoid factors (RF), and newly discovered autoantibodies whose role in pSjD is still unknown (3).

Antibodies against poly-U-binding factor 60 kDa (PUF60) protein have been recently identified in pSjD and dermatomyositis (DM) patients. In pSjD, these antibodies were associated with anti-Ro52 antibodies, RF, and hyperglobulinaemia. Still interestingly, they also had a higher prevalence of skin ulcerations and increased disease activity levels over time in DM (4). PUF60 is a nucleic acid-binding protein, also known as RoBPI (Ro RNP-binding protein), catalysing the initial phase of spliceosome assembly through the interaction with specific ribonucleoproteins (RNPs), such as Ro60, which contributes to small RNAs quality control. However, the precise clinical significance of anti-PUF60 antibodies in pSjD pathogenesis is incompletely understood (5).

A master regulator of the physiological secretory process, known to be abnormally distributed and expressed in pSjD patients, is aquaporin-5 (AQP5). This small integral membrane protein allows the diffusion of water and small solutes by forming highly selective pores in the apical membrane of acinar cells of salivary glands (6). Dysfunctional AQP5 proteins in pSjD patients may contribute to decreased salivary secretion, and it has been hypothesised that anti-AQP5 antibodies may contribute to this secretive impairment. The experimental induction of anti-AQP5 antibodies in mouse models leads to sialadenitis and reduced salivary flow (7). Recently, it was suggested that anti-AQP5 antibodies are specific to pSjD patients and linked to the presence of other autoantibodies, including anti-SSA, RF, and ANA, but also to histopathologic features of pSjD. However, the relationship with the systemic manifestations of pSjD has not been investigated so far (8).

Therefore, the primary endpoint of this study was to assess the circulating levels of anti-AQP5 and anti-PUF60 antibodies in Italian pSjD patients and, secondly, to investigate potential correlations of these antibodies with clinical and laboratory features such as disease activity and symptoms of patients in pSjD.

Materials and methods

Patients

Patients with a diagnosis of pSjD according to the 2016 ACR-EULAR Classification Criteria for pSjD (9), consecutively examined at the Rheumatology Outpatient Clinic of the University of Bari "Aldo Moro" and giving their consent for the study were enrolled and cross-sectionally evaluated. Age- and sex-matched healthy donors (HD), without any sicca symptom, were selected as a control group. Demographic and clinical data were recorded, including age (years), sex, disease duration (months), disease activity according to EULAR Sjögren's syndrome disease activity index (ESSDAI) (10) and subjective symptoms assessment via the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (11).

The ESSDAI is a EULAR-validated outcome measure used for the objective clinical evaluation of disease activity in patients with pSjD, developed to pro-

vide a standardised assessment of organ systems and clinical manifestations associated with pSjD. The ESSDAI score is calculated based on the presence and severity of clinical manifestations in 12 domains, including constitutional, lymphadenopathy, glandular, articular, cutaneous, pulmonary, renal, muscular, peripheral nervous system, central nervous system, haematological, and biological domains, each representing a different aspect of the disease and contributing to the ESSDAI score (12). The ESSPRI is a simple patient-reported outcome measure (PROM), designed to assess patient-reported symptoms and their impact on individuals with pSjD. Unlike the ESSDAI, which focuses on clinical and objective measures of disease activity, the ESSPRI is centered around the patient's subjective perception of symptoms, including dryness, fatigue, and pain in the last two weeks on a visual analogue scale (VAS) from 0 to 10. Together with the ESSDAI, the ESSPRI provides a more comprehensive assessment of pSjD by considering both clinical-objective and patient-subjective evaluations. According to the ESSDAI assessment, the visiting physician considers a patient to have high disease activity when the score is ≥14. At the same time, an ESSPRI score ≥5 is the cut-off for high severity of symptoms, as perceived by the patient (13).

The clinical assessment encompassed data regarding sicca symptoms, but also the presence of salivary gland enlargement or development of extraglandular manifestations. The laboratory panel included full blood count (FBC), liver and kidney function tests, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antinuclear antibodies (ANA), anti-Ro/SSA and/or anti-La/SSB autoantibodies, complement C3 and C4 fractions, rheumatoid factor (RF) and a qualitative cryoglobulinemia test. Ongoing treatments were recorded, including corticosteroids (CCs), immunosuppressive agents, and pilocarpine.

This study complied with the ethical guidelines of the 1975 Helsinki's Declaration and was approved by the local ethics committee (Ethics Review Board of the Bari Policlinico, comitatoetico@policlinico.ba.it, protocol no. 5277). All enrolled subjects gave written informed consent to participate with explicit personal data protection.

AQP5 and PUF60 antibodies detection

Blood samples from pSjD patients and HD were collected in tubes during clinical evaluation and, after clot formation at room temperature for at least 15 minutes, was centrifuged for 10 minutes at 2000 Relative Centrifugal Force (RCF). Serum aliquots were stored at -30°C until use. The presence of antibodies against AQP5 and PUF60 proteins was assayed by commercially available immunological tests using the two-site sandwich ELISA method. The PUF60 ELISA kit (MBS280488, species: Human, MyBiosource, Southern California, San Diego, USA) and the AQP5 ELISA kit (SEA583Hu, humans, Cloud-Clone, Katy, Texas, USA) were used for quantitative measurement of human PUF60 and AQP5 antibodies in our study samples, respectively.

Briefly, according to the manufacturer's instructions, the serum samples and standards were appropriately diluted and incubated in duplicate for 2 hours at 37°C in 96-well microplates. Then, after the required washes, 100 µL of biotin conjugate (1 x) was added to each well for a further 1-hour incubation at 37°C. Again, after washing in triplicate with fresh wash buffer and its complete removal by aspiration, 100 µL of Streptavidin- horseradish peroxidase (HRP) (1 x) was added to each well and incubated for 1 hour at 37°C. After the last cycle of three washes, 100 µL of substrate solution was added to each well and incubated again for 15 minutes at 37°C in the dark. After adding 50 µL of stop solution to each well, the optical density of each well was read using a reader set at 450 nm and 540 nm. Readings at 540 nm were subtracted from those at 450 nm to correct for optical plate imperfections and improve accuracy. The average of duplicate readings for each standard and sample was recorded, and the average of zero standard optical density was subtracted. After creating a standard curve by generating a four-parameter logistic curve fit, the data were obtained by plotting the standard curve or the samples for AQP5 or PUF60 antibody concentrations.

Positive antibody status was attributed if a concentration greater than 2 standard deviations (SD) of the mean HD value was detected.

Statistical analysis

Data are expressed as mean and SD, median and interquartile range (IQR), or number and percentage, when appropriate. The distribution of data was assessed with the Shapiro-Wilk test. Differences between continuous variables were evaluated using the Mann-Whitney test, while Fisher's probability test was used for differences between categorical data.

Odds ratios (OR) of high disease activity, defined as ESSDAI ≥14, were estimated via adjusted logistic regression analysis. Of the available variables, 4 were included in the final adjusted model as confounders of interest: disease duration (months), age (years), oral corticosteroid (CCS) therapy (yes or no), and elevated ESSPRI level (score ≥5). Variables investigated in the regression model were anti-AQP5 level, anti-PUF60 level, presence of anti-SSA antibodies, presence of anti-SSB antibodies, RF positivity, reduction of C3 (>90 mg/dL) and C4 (>10 mg/dL) complement fractions, increase of inflammatory markers (ESR >20 mm/h and CRP >2.9 mg/L), and presence of cryoglobulinaemia.

The results are presented as OR with 95% confidence intervals (CI). For all tests, a p-value <0.05 was considered statistically significant. Data were analysed using GraphPad Prism software (v. 9.5.1).

Results

Serum samples from 36 pSjD patients and 8 HD were analysed (Table I). All recruited subjects were female. The mean age ± SD among pSjD patients in our cohort was 53 years old ± 11 years, with a median (IQR) disease duration of 24 months (6–74 months). Anti-AQP5 and anti-PUF60 levels did not significantly differ between pSjD patients and HD. The mean ± SD serum anti-AQP5 level was 986±156 pg/ml in pSjD patients and 933±210 ng/ml in HD (p=0.45), while the mean ± SD serum anti-PUF60 level was 225±76 pg/

Table I. Features of pSjD patients and HD, compared according to disease activity state expressed as low-moderate or high ESSDAI.

Characteristics	SS patients (n.36)	HD (n.8)	ESSDAI < 14 (n.26)	ESSDAI ≥ 14 (n.10)	*p*-value
Age (years) ± SD	53 ± 11	54 ± 8	55 ± 10	49 ± 14	0.22
Female n. (%)	36 (100)	8 (100)	26 (100)	10 (100)	0.99
Anti-AQP5 (pg/mL) median (IQR)	765 (592 - 1138)	960 (840 - 1110)	675 (570 - 862)	1365 (815-1835)	**0.0008***
Anti-AQP5 positive n. (%)	5 (13.9)	**0 (0)**	0 (0)	5 (50)	**0.0007***
Anti-PUF60 (pg/mL) median (IQR)	47.0 (20.7 – 206.8)	51.0 (16.0 – 77.0)	44.5 (16.0 – 205.5)	51.0 (43.5-234.3)	0.37*
Anti-PUF60 positive n. (%)	10 (27.8)	**1 (12.5)**	7 (26.9)	3 (30)	0.99*
Disease duration (months) median IQR	24 (6 - 74)		26 (10 - 85)	6 (3 - 25)	**0.048**
ESSPRI ≥ 5 n. (%)	14 (38.9)		7 (26.9)	7 (70)	**0.026**
ANA positive n. (%)	36 (100)		26 (100)	10 (100)	0.99
anti-SSA positive n. (%)	31 (86.1)		22 (84.6)	9 (90)	0.99
anti-SSB positive n. (%)	18 (50)		14 (53.8)	4 (40)	0.71
RF > 15 UI/ml n. (%)	14 (38.9)		10 (38.5)	4 (40)	0.99
C3 < 90 mg/d n. (%)	7 (19.4)		5 (19.2)	2 (20)	0.99
C4 < 10 mg/dL n. (%)	5 (13.8)		2 (7.7)	3 (30)	0.12
ESR > 20 mm/ n. (%)	20 (55.6)		14 (53.8)	6 (60)	0.99
CRP >2.9 mg/L n. (%)	12 (33.3)		6 (23.1)	6 (60)	0.053
Cryoglobulinaemia n. (%)	7 (19.4)		2 (7.7)	5 (50)	**0.01**
WBC <3.000/mm^3 n. (%)	7 (19.4)		5 (19.2)	2 (20)	0.99
Joint involvement n. (%)	25 (69.4)		16 (61.5)	9 (90)	0.13
Skin manifestations n. (%)	7 (19.4)		4 (15.4)	3 (30)	0.37
Kidney involvement n. (%)	3 (8.3)		2 (7.7)	1 (10)	0.99
Pilocarpine treatment n. (%)	6 (16.7)		4 (15.4)	2 (20)	0.99
CS treatment n. (%)	19 (52.8)		13 (50)	6 (60)	0.72
HCQ treatment n. (%)	15 (41.7)		10 (38.5)	5 (50)	0.71
MTX treatment n. (%)	6 (16.7)		4 (15.4)	2 (20)	0.99
RTX treatment n. (%)	3 (8.3)		0 (0)	3 (30)	**0.02**
Xerostomia n. (%)	34 (94.4)		25 (96.1)	9 (90)	0.48
Xeropthalmia n. (%)	33 (91.7)		24 (92.3)	9 (90)	0.99
Schirmer test positive n. (%)	33 (91.7)		24 (92.3)	9 (90)	0.99
Focus score (0-4) median IQR	4 (1 - 4)		4 (1 - 4)	4 (3 - 4)	0.61

(* ESSDAI <14 *vs*. >14)
pSjD: primary Sjögren's disease; HD: healthy donors; SD, standard deviation; IQR: interquartile range; anti-AQP5: anti-aquaporin 5; anti-PUF60: anti-poly-U-binding factor 60 kDa protein; ESSDAI: EULAR Sjögren's syndrome disease activity; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; ANA: antinuclear antibodies; anti-SSA: anti-Sjögren's-syndrome-related antigen A (Ro) autoantibodies; anti-SSB: anti-Sjögren's-syndrome-related antigen B (La) autoantibodies; RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; WBC: white blood cells; CS: corticosteroids; HCQ: hydroxychloroquine; MTX: methotrexate; RTX: rituximab; NA: not assessable.
p-value >0.05, comparing SS patients with high *vs*. low-moderate ESSDAI.

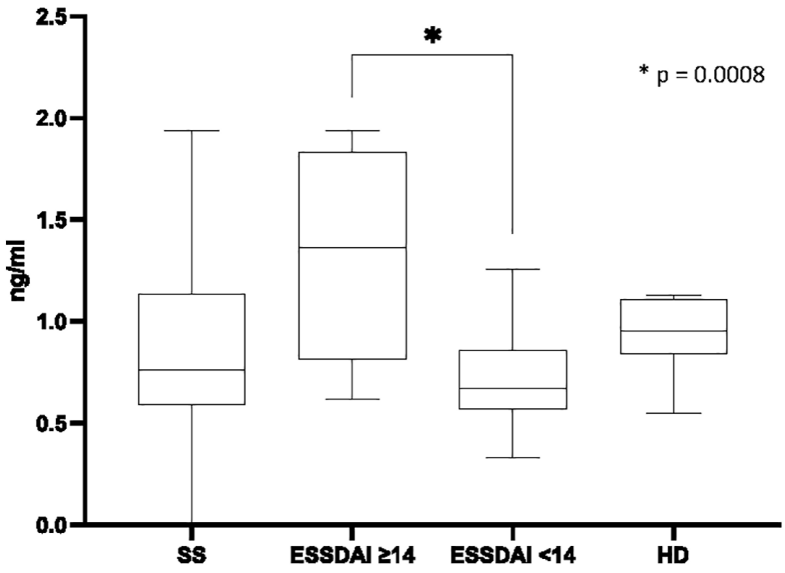

Fig. 1. Anti-AQP5 antibodies level in HD and pSjD patients, classified as low-moderate or high disease activity state according to ESSDAI.
pSjD: primary Sjögren's disease; anti-AQP5: anti-aquaporin 5; ESSDAI: EULAR Sjögren's syndrome disease activity; High: ESSDAI ≥14; Low: ESSDAI <14.

ml and 75±31 pg/ml (p=0.75), in patients with pSjD and HD, respectively. Considering the positivity status for anti-AQP5 (defined cut-off: 1353 pg/ml) and anti-PUF60 (defined cut-off: 137 pg/ml), 5 out of 36 (13.9%) pSjD patients and none of HD were anti-AQP5 positive (p=0.56), while 10 out of 36 (27.8%) pSjD patients and 1 HD were anti-PUF60 positive (p=0.65).
However, anti-AQP5 level (p>0.001) and anti-PUF60 level (p>0.0001) were significantly associated with elevated ESSPRI (≥5) in our cohort. Furthermore, a significant association of anti-AQP5 antibody levels with the presence of RF (p>0.0001), anti-SSB (p>0.01), and ANA (p>0.05), but not anti-SSA antibodies, was observed. Similarly, anti-PUF60 antibody levels were significantly associated with RF (p>0.0001), anti-SSB (p>0.0001), and

Table II. Unadjusted and adjusted odds ratios (OR) of high disease activity state (ESSDAI ≥14) by logistic regression analysis.

Covariates	Unadjusted		Adjusted	
	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Anti-AQP5 level	**49.2 (2.9 – 82.4)**	**0.007**	**128.9 (2.7 – 615.0)**	**0.014**
Anti-PUF60 level	1.0 (1.0 - 1.0)	0.736	1.0 (0.9 - 1.0)	0.638
Anti-SSA positive	1.6 (0.2 - 16.7)	0.678	2.3 (0.2 - 31.6)	0.531
Anti-SSB positive	0.6 (0.2 - 2.5)	0.459	0.5 (0.1 - 3.1)	0.475
RF > 15 UI/ml	1.1 (0.2 - 4.7)	0.932	0.4 (0.1 - 2.9)	0.364
C3 < 90 mg/dL	1.0 (0.2 - 6.5)	0.958	1.9 (0.2 - 19.3)	0.603
C4 < 10 mg/dL	5.1 (0.7 – 37.1)	0.105	**60.0 (1.1 – 318.9)**	**0.043**
ESR >20 mm/h	1.3 0.3 – 5.6)	0.740	1.7 (0.3 – 10.6)	0.557
CRP >2.9 mg/L	**5.0 (1.1 – 23.8)**	**0.043**	**12.9 (1.2 – 137.2)**	**0.034**
Cryoglobulinaemia	12.0 (1.8 – 80.4)	0.10	42.4 (0.8 – 218.2)	0.062

Anti-AQP5: anti-aquaporin 5; anti-PUF60: anti-poly-U-binding factor 60 kDa protein; ESSDAI: EULAR Sjögren's syndrome disease activity; anti-SSA: anti-Sjögren's-syndrome-related antigen A (Ro) autoantibodies; anti-SSB: anti-Sjögren's-syndrome-related antigen B (La) autoantibodies; RF: rheumatoid factor; C3: complement component 3; C4: complement component 4; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

ANA (p>0.05) presence. Still, unlike anti-AQP5 antibodies, an association was noted between anti-PUF60 antibodies and the presence of anti-SSA antibodies (p>0.0001).

Stratifying pSjD patients according to ESSDAI disease activity score, 10 patients (27.8%) had an ESSDAI ≥14 defined as high disease activity, 1 (2.8%) had an ESSDAI <14 and >5 expressing moderate disease activity, and 25 (69.4%) had an ESSDAI <5, considered low disease activity. Patients with high disease activity had significantly higher anti-AQP5 antibody titres (p=0.0008) than those with low-moderate disease activity (Fig. 1). On the contrary, anti-PUF60 antibodies titres, even after stratifying patients according to ESSDAI into high and low-moderate disease activity, were not significantly different between groups (p=0.37).

In our cohort, pSjD patients with high disease activity (ESSDAI ≥14) had significantly shorter disease duration (p>0.05), higher prevalence of cryoglobulins (p>0.01), more frequently an ESSPRI ≥5 (p>0.03) and elevated CRP levels (p=0.05) compared to pSjD patients with ESSDAI <14. No relevant correlation with anti-PUF60 antibody levels could be detected.

Therapy with disease-modifying antirheumatic drugs (DMARDs) or oral CCS was administered in 26 (72.2%) patients. Specifically, 19 (52.7%) patients were on CCS, 15 (41.7%) received hydroxychloroquine, 6 (16.7%) methotrexate, and 3 (8.3%) were on rituximab; pilocarpine was administered in 6 (16.7%) patients. Only treatment with rituximab was significantly associated with a high disease activity state, expressed as ESSDAI ≥14 (p>0.02).

On the contrary, a focus score at salivary gland biopsy, the presence of xerostomia or xeropthalmia, and positivity to the Schirmer test were not associated with an elevated ESSDAI.

At unadjusted logistic regression analysis, anti-AQP5 and CRP levels were able to predict those pSjD patients at high disease activity state (ESSDAI ≥14), with an OR of 49.2 (95%CI 2.9-82.4; p=0.007) and 5.0 (95%CI 1.1-23.8; p=0.043), respectively. After adjustment for disease duration, age, oral CCS treatment, and ESSPRI level, anti-AQP5 and CRP levels were confirmed as predictors of high disease activity, with an OR of 128.9 (95%CI 2.7-615.0; p=0.014) and OR of 12.9 (95%CI 1.2-137.2; p=0.034), respectively. Moreover, a low C4 complement fraction was a statistically significant predictor of a high disease activity state (OR 60.0 - 95%CI 1.1-318.9; p=0.043) (Table II).

Discussion

Our pilot study showed that the level of anti-AQP5 antibodies was associated with a high disease activity state according to ESSDAI and subjectively increased symptom severity expressed via ESSPRI. Similarly, the level of anti-PUF60 antibodies was associated with increased ESSPRI, but no association with ESSDAI was observed.

Identifying disease-specific biomarkers is a clinical challenge, particularly for pSjD, where high variability of clinical manifestations may hamper disease activity and therapeutic outcomes, which are evaluated mainly by patients' reported symptoms (14). Searching for specific biomarkers correlating with pSjD disease activity has become extremely important in recent years. A significant example is the positioning of Ro (SSA) and La (SSB) autoantibodies in the American-European Consensus Criteria classification criteria for pSjD, with considerable value as a diagnostic tool in clinical practice (15).

In our cohort, 31 pSjD patients (86.0%) presented positivity for anti-SSA antibodies and 17 (47.0%) for anti-SSB antibodies, but in both cases, no relationships with ESSDAI or ESSPRI were observed. Interestingly, the levels of anti-AQP5 and anti-PUF60 antibodies were associated with other autoantibodies, including RF, anti-SSB, and ANA, while the level of anti-PUF60 antibodies was also related to the presence of anti-SSA.

We detected a significant association of anti-AQP5 antibody levels with ESSDAI and ESSPRI. The fact that AQP5 plays a key role in regulating exocrine glands' secretive function by controlling water fluxes through cell membranes has been demonstrated in animal models. Experimental studies investigating the molecular mechanisms of radiation-induced xerostomia in irradiated rat submandibular glands confirmed the hypothesis that down-regulation of AQP5 expression is one of the mechanisms of radiation-induced xerostomia, concluding that irradiation significantly decreases AQP5 level in submandibular glands, the latter being essential in saliva flow (16, 17). More recently, it has been demonstrated that the experimental induction of anti-AQP5 antibodies by molecular mimicry in animal models leads to decreased salivary flow. Anti-AQP5 antibodies in mouse models affected AQP5 trafficking, interfering with the salivary secretive process. It was shown that mice positive for the human/mouse anti-AQP5 autoantibody had reduced

salivary flow rates and that the salivary flow rate tended to decrease with increased anti-AQP5 IgG levels. The presence of anti-AQP5 autoantibodies was associated with a low salivary flow rate in mice, suggesting the role of anti-AQP5 autoantibodies in the dryness of pSjD patients. Of note, despite the reduced salivary flow, no histologic abnormality was observed in the salivary glands of the mice with anti-AQP5 autoantibodies, suggesting a functional impairment rather than tissue damage as the main pathogenetic mechanism (7).

Although the principal cause of exocrine glands hypofunction remains unclear in pSjD, likely inflammation, acini destruction, and altered AQP5 expression and/or localisation are involved. Coherently, a link between inflammation and altered AQP5 expression, mediated by cytokines such as interferon-γ and tumour necrosis factor α, has been proposed (18, 19). Anti-AQP5 autoantibodies presence was evaluated in an Asian multicentric cohort including 111 pSjD patients and 43 non-SjD "SICCA" controls. A strong correlation with the presence of other autoantibodies, including SSA, RF, and ANA, but also with histopathologic and clinical features of pSjD such as focal lymphocytic score (FLS) ≥1 and ocular staining score ≥3 was observed (8).

The association of high anti-AQP5 levels with high FLS suggested that local production at the salivary glands contributes to the levels of anti-AQP5 autoantibodies. Still, the correlation between the presence of these autoantibodies and the systemic manifestations of pSjD, ESSPRI, and ESSDAI were not investigated (20). In our cohort, a high disease activity state (ESSDAI ≥14) was significantly associated with higher anti-AQP5 levels, elevated ESSPRI, and lower disease duration. Surprisingly, no association between elevated ESSDAI and the presence of xerostomia, xerophthalmia, including positivity to the Schirmer test, and focus score obtained from salivary gland biopsy were observed in our cohort.

According to the literature, apart from previously reported data on the presence of anti-PUF60 antibodies in DM and pSjD patients, existing evidence on anti-PUF60 antibodies is scarce. Overexpression of abnormal splicing variants of the PUF60 gene has been associated with colorectal cancer development and detected in the sera of patients with oesophageal squamous cell carcinoma, potentially acting as a new biomarker (21, 22).

In our cohort, the level of anti-PUF60 antibodies was associated with elevated ESSPRI and other autoantibodies, including RF, anti-SSA, anti-SSA, and ANA. However, no significant association with ESSDAI was observed.

The main limitation of this study was the small sample size, specifically the low number of included HD and the absence of "sicca" controls. Furthermore, all enrolled subjects were female, as pSjD predominantly affects females. Therefore, the impact of sex on disease activity status and autoantibody levels could not be assessed. Finally, all patients treated with rituximab were also affected by cryoglobulinaemia.

Despite this study's limitations, our findings give valuable insights into the clinical meaning of anti-AQP5 and anti-PFU60 antibodies in patients affected by pSjD. The combination of patient-reported outcomes, such as the ESSPRI, and objective measures of disease activity, such as the ESSDAI, provided a comprehensive approach to assessing pSjD and highlighted the relevant role of novel biomarkers in the view of new therapeutic development. Future clinical trials with composite endpoints and larger cohorts of patients with pSjD (23) may confirm our preliminary findings.

Take home messages

- In primary Sjögren's disease (pSjD), in addition to glandular inflammation and atrophy, functional secretion impairment may contribute to dryness.
- Aquaporin-5 (AQP5) plays a key role in gland secretion, and abnormal distribution of AQP5 proteins and the presence of anti-AQP5 antibodies have been reported in pSjD.
- Anti-AQP5 antibodies are detectable and their levels, but not anti-Poly-U-binding-Factor-60 (PUF60) antibodies, correlate with clinical and laboratory features of pSjD.

References

1. NEGRINI S, EMMI G, GRECO M *et al*.: Sjögren's syndrome: a systemic autoimmune disease. *Clin Exp Med* 2022; 22(1): 9-25. https://doi.org/10.1007/s10238-021-00728-6
2. DINESCU ŞC, FORŢOFOIU MC, BUMBEA AM, CIUREA PL, BUSUIOC CJ, MUŞETESCU AE: Histopathological and immunohistochemical profile in primary Sjögren's syndrome. *Rom J Morphol Embryol* 2017; 58(2): 409-17.
3. OGAWA Y, TAKEUCHI T, TSUBOTA K: Autoimmune epithelitis and chronic inflammation in Sjögren's syndrome-related dry eye disease. *Int J Mol Sci* 2021; 22(21): 11820. https://doi.org/10.3390/ijms222111820
4. FIORENTINO DF, PRESBY M, BAER AN *et al*.: PUF60: a prominent new target of the autoimmune response in dermatomyositis and Sjögren's syndrome. *Ann Rheum Dis* 2016; 75(6): 1145-51. https://doi.org/10.1136/annrheumdis-2015-207509
5. ZHANG YM, YANG HB, SHI JL *et al*.: The prevalence and clinical significance of anti-PUF60 antibodies in patients with idiopathic inflammatory myopathy. *Clin Rheumatol* 2018; 37(6): 1573-80. https://doi.org/10.1007/s10067-018-4031-4
6. HOSOI K, YAO C, HASEGAWA T, YOSHIMURA H, AKAMATSU T: Dynamics of salivary gland AQP5 under normal and pathologic conditions. *IJMS* 2020; 21(4): 1182. https://doi.org/10.3390/ijms21041182
7. LEE A, YOO DK, LEE Y *et al*.: Induction of anti-aquaporin 5 autoantibody production by immunization with a peptide derived from the aquaporin of prevotella melaninogenica leads to reduced salivary flow in mice. *Immune Netw* 2021; 21(5): e34. https://doi.org/10.4110/in.2021.21.e34
8. JEON S, LEE J, PARK SH, KIM HD, CHOI Y: Associations of anti-aquaporin 5 autoantibodies with serologic and histopathological features of Sjögren's syndrome. *J Clin Med* 2019; 8(11): E1863. https://doi.org/10.3390/jcm8111863
9. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017; 69(1): 35-45. https://doi.org/10.1002/art.39859
10. SEROR R, BOWMAN SJ, BRITO-ZERON P *et al*.: EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. *RMD Open* 2015; 1(1): e000022. https://doi.org/10.1136/rmdopen-2014-000022
11. SEROR R, RAVAUD P, MARIETTE X *et al*.: EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI): development of a consensus patient index for primary Sjogren's syndrome. *Ann Rheum Dis* 2011; 70(6): 968-72. https://doi.org/10.1136/ard.2010.143743
12. DE WOLFF L, ARENDS S, VAN NIMWEGEN JF, BOOTSMA H: Ten years of the ESSDAI: is it fit for purpose? *Clin Exp Rheumatol* 2020; 38 (Suppl. 126): S283-90.
13. SEROR R, BOOTSMA H, SARAUX A *et al*.: Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary

Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). *Ann Rheum Dis* 2016; 75(2): 382-9. https://doi.org/10.1136/annrheumdis-2014-206008

14. TIAN Y, YANG H, LIU N, LI Y, CHEN J: Advances in pathogenesis of Sjögren's syndrome. *J Immunol Res* 2021; 2021: 5928232. https://doi.org/10.1155/2021/5928232
15. VEENBERGEN S, KOZMAR A, VAN DAELE PLA, SCHREURS MWJ: Autoantibodies in Sjögren's syndrome and its classification criteria. *J Transl Autoimmun* 2022; 5: 100138. https://doi.org/10.1016/j.jtauto.2021.100138
16. TAKAKURA K, TAKAKI S, TAKEDA I *et al*.: Effect of cevimeline on radiation-induced salivary gland dysfunction and AQP5 in submandibular gland in mice. *Bull Tokyo Dent Coll* 2007; 48(2): 47-56. https://doi.org/10.2209/tdcpublication.48.47
17. LI Z, ZHAO D, GONG B *et al*.: Decreased saliva secretion and down-regulation of AQP5 in submandibular gland in irradiated rats. *Radiat Res* 2006; 165(6): 678-87. https://doi.org/10.1667/rr3569.1
18. SOYFOO MS, DE VRIESE C, DEBAIX H *et al*.: Modified aquaporin 5 expression and distribution in submandibular glands from NOD mice displaying autoimmune exocrinopathy. *Arthritis Rheum* 2007; 56(8): 2566-74. https://doi.org/10.1002/art.22826
19. SOYFOO M, KONNO A, BOLAKY N *et al*.: Link between inflammation and aquaporin-5 distribution in submandibular gland in Sjögren's syndrome? *Oral Dis* 2012; 18(6): 568-74. https://doi.org/10.1111/j.1601-0825.2012.01909.x
20. ALAM J, KOH JH, KIM N *et al*.: Detection of autoantibodies against aquaporin-5 in the sera of patients with primary Sjögren's syndrome. *Immunol Res* 2016; 64(4): 848-56. https://doi.org/10.1007/s12026-016-8786-x
21. KOBAYASHI S, HOSHINO T, HIWASA T *et al*.: Anti-FIRs (PUF60) auto-antibodies are detected in the sera of early-stage colon cancer patients. *Oncotarget* 2016; 7(50): 82493-503. https://doi.org/10.18632/oncotarget.12696
22. KOBAYASHI S, HIWASA T, ISHIGE T *et al*.: Anti-FIRΔexon2, a splicing variant form of PUF60, autoantibody is detected in the sera of esophageal squamous cell carcinoma. *Cancer Sci* 2019; 110(6): 2004-13. https://doi.org/10.1111/cas.14024
23. LONGHINO S, CHATZIS LG, DAL POZZOLO R *et al*.: Sjögren's syndrome: one year in review 2023. *Clin Exp Rheumatol* 2023; 41(12): 2343-56. https://doi.org/10.55563/clinexprheumatol/255qsx